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13. ABSTRACT (Maximum 200 Words) Purpose and scope: A large genetic association study is being conducted, to examine relationships of prostate cancer risk with polymorphic variation in a series of selected candidate genes that are involved in pathways determining the synthesis of IGF-I and IGF-binding proteins, as well as biological response to IGF-I. The study will be performed within a large Swedish case-control study ("CAPS"). Progress report: We completed, as planned, the recruitment of prostate cancer cases and control subjects, biobanking of blood samples, extraction of DNA samples and shipment of DNA aliquots to the laboratory (IARC) where genotyping of single nucleotide polymorphisms will be performed. The shipment of plasma samples, for measurement of IGF-I and IGFBP-3, was postponed for practical reasons, until year 2 of the project. Conclusions: Our project is entirely on schedule, and will start producing its first scientific results on SNP genotyping and statistical association analyses in year 2, as planned.				
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INTRODUCTION

Evidence is rapidly accumulating that insulin-like growth factor-I (IGF-I) can enhance the development of tumors in different organs. Studies *in vitro* have shown that IGF-I inhibits apoptosis and stimulates cell proliferation in a wide variety of cell types. Furthermore, tumor development can be strongly enhanced in animals or organs that have been genetically or otherwise manipulated to either overexpress IGF-I or the IGF-I receptor, whereas animals made deficient in IGF-I are protected. Experiments with IGF-I^{-/-} null mice have shown that normal IGF-I levels are required for prostate gland development, and transgenic mice expressing human *IGF1* in basal epithelial cells of the prostate have a high spontaneous incidence of prostatic tumors. In men, several prospective cohort studies and case-control studies have shown an increased prostate cancer risk among men who have elevated plasma IGF-I levels – expressed either as absolute concentrations, or relative to levels of IGFBP-3, IGF's major plasmatic binding protein.

Most of IGF-I and IGF-binding proteins in the circulation originates from the liver, but all peptides are also formed in other organs, including the prostate, where they exert paracrine and autocrine effects. Circulating IGF-I, as an endocrine factor, can diffuse towards its target tissues. In addition, IGF-I synthesis by the liver and many other organs is very much controlled by the same endocrine and nutritional factors. The major endocrine stimulus to IGF-I synthesis, in liver and many other tissues, is provided by growth hormone (GH). Thus, elevated IGF-I in blood most likely reflects an elevated pituitary GH secretion, and most likely indicates also elevated levels in other tissues where GH also provides the principal stimulus to IGF-I synthesis.

Given the increasing evidence that elevated IGF-I may enhance cancer development, it is important to understand what factors can lead to elevated IGF-I in the circulation and tissues. Besides nutritional status (Kaaks & Lukanova, 2001), heritability studies have shown that, at least in western, well-nourished populations, a large part (40-60 %) of variation in IGF-I is (co-) determined by genetic factors (Hong et al., 1996; Harrela et al., 1996; Verhaeghe et al., 1996). So far, however, no studies have been published, reporting a comprehensive search for polymorphisms in a full panel of genes involved in regulating IGF-I synthesis, and correlating such a panel with inter-subject variations in IGF-I and IGFBP-3 levels.

Besides the genes for IGF-I (*IGF1*) and IGFBP-3 (*IGFBP3*), major candidate genes to be examined are those involved in the pituitary release or biological action of growth hormone – the primary physiological stimulus for the synthesis of both IGF-I and IGFBP-3. This latter includes the genes for somatostatin (*SST*) and its receptors (*SSTR1-5*), pituitary-specific transcription factor (or POU-domain class 1 transcription factor 1 (*POU1F1*); growth hormone (*GH1*) and its receptor (*GHR*), growth hormone releasing hormone (*GHRH*), and the GHRH receptor (*GHRHR*). Ghrelin (*GHRL*), a recently identified new peptide hormone produced by endocrine cells in the stomach, also stimulates growth hormone secretion. It is the first identified natural ligand for a previously cloned growth hormone secretagogue receptor (*GHSR*) which is present in the pituitary gland and the hypothalamic region of the brain. In the circulation, IGF-I and a large percentage of IGFBP-3 are bound to a third peptide, referred to as Acid Labile Subunit (*IGFALS*) which has a key role in stabilizing the circulating pool of these peptides, and in regulating IGF-I release towards tissues. For each of these genes, polymorphisms that change gene expression or protein function can be expected to result in a relative increase or decrease in circulating IGF-I or IGFBP-3 levels.

The specific aims of our project are the following:

- to examine associations of prostate cancer risk with polymorphic variants (single nucleotide polymorphisms [SNPs] or their haplotypes) of selected candidate genes that may determine the synthesis and circulating levels of IGF-I, and/or biological response to IGF-I;

- to confirm that elevated IGF-I levels, as absolute concentrations or expressed relative to concentrations of IGFBP-3, are associated with an increased risk of prostate cancer; and
- examine whether associations of prostate cancer risk with polymorphic gene variants can be explained, at least in part, by associations of the same gene variants with circulating IGF-I or IGFBP-3 levels.

Table 1. Candidate genes for studies of association with plasma IGF-I, IGFBP-3, and prostate cancer risk.

Gene	Name and Function of gene product
IGF-I	Insulin-like growth factor-I
GH1	Growth hormone: Main stimulus for synthesis of IGF-I and IGFBP-3
GHR	Growth hormone receptor: mediates GH effects
GHRH	Growth hormone releasing hormone: stimulates pituitary GH release
GHRHR	Growth hormone releasing hormone receptor; Mediates GHRH effects
SST	Somatostatin; inhibits pituitary GH release
SSTR1 – SSTR5	Somatostatin receptors, types 1 - 5; mediate SST effects on pituitary GH release
POU1F1	Pituitary-specific transcription factor; crucial for pituitary GH synthesis
IGF1R	IGF-I receptor
GHRL	Ghrelin
GHSR	Growth hormone secretagogue receptor
IGFALS	IGF binding protein, acid labile subunit
IGFBP1 - 6	IGF-binding proteins 1 to 6

BODY

For year 1, our tasks, as in the "Statement of Work" of our original grant application, were the following:

Task 1 (months 1-6): Completion of the recruitment of prostate cancer cases and control subjects into the Swedish "CAPS" (Cancer of the Prostate, Sweden) project, using suitable matching and selection criteria for the control subjects; Storage of blood samples (plasma and buffy coats) in the Medical Biobank at Umeå University;

This objective was entirely achieved, and actually even exceeded: A total of 2831 prostate cancer cases (57% of which were localized tumors, and 43% locally advanced tumors) and 1784 control subjects were recruited into the CAPS project, and from these subjects questionnaire data and blood samples were collected as planned. Blood samples were fractionated into plasma and buffy coats, and stored in the Umeå Medical Biobank. The increase in numbers of prostate cancer cases and control subjects was motivated by the fact that the speed of subject recruitment could be accelerated (thus allowing a cost-effective extension of study size), plus the consideration that the sample size initially foreseen (1200 cases and 1200 control subjects) might have provided insufficient statistical power to examine associations of genetic polymorphisms with prostate cancer risk, by subsets of different tumor grade and stage (e.g., local vs. advanced tumors).

Task 2 (months 6-12): Retrieval of plasma samples from the Medical Biobank, assembly of plasma samples into batches of case-control sets for immunoassay of IGF-I and IGFBP-3;

This task was postponed to year 2 of the project, because of some changes in the agenda of the Hormones and Cancer Laboratory where the assays of IGF-I and IGFBP-3 will be

performed, and because we have to liberate freezer space at IARC where the plasma aliquots will be stored.

Task 3 (months 4-15): DNA extraction from buffy coat samples of all prostate cancer cases and control subjects (total of 2400 subjects originally foreseen); and

This task was fully completed: DNA was extracted from the buffy coats of all 2831 prostate cancer cases, and 1784 controls.

Task 4 (months 4-15): Preparation of microwell plates with DNA aliquots for genotyping of genetic polymorphisms, at IARC.

This task was also entirely completed: 500 ng. aliquots of DNA were distributed into microtiter plates and shipped to IARC (shipped in May 2004). The DNA plates sent to IARC are in a format that will allow immediate preparation of microwell plates for PCR amplification and determination of specific single nucleotide polymorphisms (SNPs) using the Taqman method.

KEY RESEARCH ACCOMPLISHMENTS

Accomplishments of year 1 include:

- completion of the recruitment of study subjects (prostate cancer cases and cancer-free control subjects), with collection of questionnaire data and blood samples;
- processing and storage of blood samples (plasma and buffy coats) in the Umeå Medical Biobank repository;
- extraction of DNA from buffy coats, and reparation of microwell plates with DNA aliquots for genotyping at IARC; and
- shipment of the DNA samples to IARC.

In addition to the above accomplishments that were planned for year 1 of this project, and which are mostly of a logistic nature, we have identified a large number of single nucleotide polymorphisms in the list of candidate genes in Table 1. Much of this work of SNP identification was already accomplished at the time we submitted our grant application for the present project, and reported as preliminary work. We have, however, identified a substantial additional number of SNPs, by searching the rapidly growing SNP databases, and through active search by systematic resequencing in the context of parallel projects. Furthermore, we have made substantial progress in the identification of 'haplotype tagging' SNPs, which allows a reduction of the total number of SNPs to be genotyped, with only minimal loss of information (due to the fact that SNPs are often in linkage disequilibrium) (Stram et al., 2003). We are therefore ready to perform extensive genotyping, for most of the candidate genes listed in Table 1, and to start performing analyses of gene-disease associations, as from year 2 of this project (as planned).

REPORTABLE OUTCOMES

The study has not yet resulted in reportable outcomes (this will be the case in years 2 and 3).

CONCLUSIONS

The recruitment of study subjects, collection of blood samples, extraction of DNA samples and shipment of DNA aliquots to the IARC have all been achieved according to schedule, and we were able to increase total numbers of case and control subjects included in the study. All is

ready for the next phase of the study (year 2), in which the risks of low- and high-grade prostate cancers risk will be related to polymorphic variation (SNPs) in the selected candidate genes involved in IGF-I metabolism and response.

REFERENCES

Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J, Toivanen L, Koskenvuo M, Leinonen P, Koistinen R, Seppala M. 1996; Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J Clin Invest.* 98:2612-5.

Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U. 1996; Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. *J Clin Endocrinol Metab.* 81:1791-7.

Schneider, Roessli & Excoffier. 2000; Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Jernstrom H, Deal C, Wilkin F, Chu W, Tao Y, Majeed N, Hudson T, Narod SA, Pollak M. 2001; Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. *Cancer Epidemiol Biomarkers Prev.* 10:377-84.

Kaaks R & Lukanova A. 2001; Energy balance and cancer: the role of insulin and IGF-I. *Proc Nutr Soc.* 60: 91-106.

Verhaeghe J, Loos R, Vlietinck R, Herck EV, van Bree R, Schutter AM. 1996; C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in cord serum of twins: genetic versus environmental regulation. *Am J Obstet Gynecol.* 175:1180-8.

Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC. 2003; Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered.* 55:27-36.

APPENDICES:

None.